

REMARKS

Claims 1 and 25 have been amended to specify that damage to cells is minimized by washing cells with a liquid wash medium. Support for this amendment can be found, for example, in claims 2, 26 and 39 as originally filed, as well as in paragraph [0031] found on page 9 of the specification.

Claims 63-81 have been canceled without prejudice to filing in a continuation application.

It is submitted that these amendments do not constitute new matter and their entry is requested.

Applicants would like to thank the Examiner and his supervisor for the courtesies extended to the undersigned and the inventor, Dr. Marie Connett-Porceddu, at the interview on 26 June 2003. During the interview Dr. Connett-Porceddu described the present invention in the context of the differences between hard and soft pines and the prior inability to regenerate transformed pine tissue of pines of the subgenus *Pinus*, i.e., hard pines, in commercially valuable quantities. According to Dr. Connett-Porceddu, one feature of the invention which enables the enhanced transformation and the regeneration of transformed embryonic hard pine tissue is the use of a liquid wash medium as opposed to the use of water to wash cells following *Agrobacterium* infection or cocultivation of embryonic hard pine tissue with *Agrobacterium*. This feature of the invention has been set forth in claims 1 and 25. The differences between hard and soft pines and the unobvious nature of plant transformation and regeneration in these species was discussed. The Examiner suggested that Declarations be submitted to address this point. Applicants are in the process of preparing Rule 132 Declarations and these will be submitted as soon as they have been executed and received by the undersigned.

The Examiner rejected claims 63-81 under 35 U.S.C. § 112, first paragraph for lack of written description. Although Applicants do not agree with this rejection, Applicants have canceled these claims in an effort to expedite the prosecution of the remaining claims without prejudice to filing in a continuation application.

The Examiner rejected claims 1-9, 11-43 and 45-81 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. (*Molecular Breeding* 5:429-440, 1999) on the basis of the disclosure

in Levee et al. of the regeneration of transformed soft pines, i.e., members of the subgenus *Strobus*. It is submitted that the presently claimed invention is not obvious from the teachings of Levee et al.

In accordance with the claimed invention, enhanced transformation and regeneration of transformed embryonic hard pine tissue is accomplished by minimizing damage to cells subsequent to *Agrobacterium* infection. It was found by the inventors that one technique to minimize damage to cells was to wash cells following *Agrobacterium* infection with a liquid wash medium. This feature is disclosed in the present specification, e.g. paragraph [0031] on page 9 and in several of the original claims. This feature has now been incorporated into claims 1 and 25, so that all of the independent claims directed to minimizing damage to cells now utilize a liquid wash medium to wash cells as a means of minimizing damage to cells subsequent to *Agrobacterium* infection. As Dr. Connett-Porceddu stated during the interview, distilled water could not be used to wash the cells, but any liquid wash medium could be used. Levee et al. uses distilled water to wash the cells of soft pines following *Agrobacterium* infection. There is no suggestion in Levee et al. to use a liquid wash medium and no suggestion that use of a liquid wash medium would result in an enhanced transformation and regeneration of transformed embryonic tissue of hard pines. Thus, it is submitted that the claimed invention is not obvious from the teachings of Levee et al.

Furthermore, Levee et al. discloses *Agrobacterium* transformation of white pine, *Pinus strobes*. As is well known in the art, white pine is a soft pine and not a hard pine. As is evident in the name, *Pinus strobes*, white pine is a member of the subgenus *Strobes* and is not a member of the subgenus *Pinus*. For example, most classifications of *Pinus* recognize two major lineages: subgenus *Strobus* (haploxylon or soft pines, with one fibrovascular bundle in the needle) and subgenus *Pinus* (diploxylon or hard pines, with two fibrovascular bundles in the needle). This division is consistent with data from wood anatomy and secondary chemistry, and is supported in recent molecular phylogenetic studies (Strauss and Doerksen, 1990, *Evolution* 44:1081-1096; Wang and Szmidt, 1993, *Plant Systematics and Evolution* 188:197-211; reviewed in Price et al., 1998, in *Ecology and Biogeography of Pinus*, Cambridge University Press, Cambridge, pp. 49-68).

Pines have a relatively rich fossil record dating back to the Early Cretaceous, 130 million years ago (review in Axelrod et al., 1986, *Ann Mo Bot Gard* **73**:565-641; Klaus et al., 1989, *Plant Systematics and Evolution* **162**:133-163; Van der Burgh, 1973, *Review of Paleobotany and Palynology*, **15**:73-275; Millar, 1993, *Ann Mo Bot Gard* **80**:471-498). The genetic distance between subgenera, at least between *Pinus* and *Strobus*, may be as large as, or larger than the genetic distance between other conifer genera, e.g., between *Cedrus* and *Abies* (Price et al., 1987, *Systematic Botany*, **12**:91-97), and if strict genetic criteria were used, they should perhaps be treated at generic rank. As is commonly known, hard pines are unable to breed with soft pines, though they can interbreed readily, if the correct timing and other conditions are provided, with other hard pine species (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C.; see also Little and Critchfield, 1969, *Subdivision of the genus Pinus pines*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C.). Hard pines are unaffected by a number of diseases, such as white pine blister rust, that readily infect soft pines. Their susceptibility to *Agrobacterium* infection appears to be quite different as well.

Levee et al. discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The amended claims are clearly directed to pine cells of the *Pinus* subgenus. It is well known to those skilled in the art that somatic embryogenesis systems for soft pines are different from those for hard pines. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as set forth in the claims. Furthermore, it is submitted that there has been no reports in the literature of the regeneration of plants following stable transformation of embryogenic cultures of

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any pines of the *Pinus* subgenus by *Agrobacterium*. In order to further establish these known differences between hard and soft pines and to provide further evidence of the nonobviousness of the present invention, Applicants are in the process of preparing Rule 132 Declarations and these will be submitted as soon as they have been executed and received by the undersigned. It is believed that these Declarations will establish that the present invention is not obvious from the teachings of Levee et al.

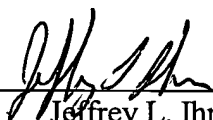
In view of the amendments to the claims and the above remarks, it is submitted that the claimed invention is not obvious from the teachings of Levee et al. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, in conjunction with the remarks made in the previous amendment, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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